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Sexual Behavior in the Guppy, *Lebistes reticulatus* (Peters).

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(Plates I-VII; Text-figures 1 &amp; 2).

## INTRODUCTION.

The guppy *Lebistes reticulatus* (Peters) is probably the best known of the poeciliid fishes. It is a viviparous fish, attractive, hardy and prolific and therefore popular with both aquarists and scientists. Recently several discussions on sexual behavior in the guppy have been included in papers primarily concerned with other aspects of behavior, morphology, physiology, genetics and taxonomy. In addition, numerous observations on this subject have appeared in the popular aquarium journals.

One of the most striking features of the sexual behavior in this species is the manner in which the males persistently pursue the females, and the great frequency with which the males jab at the genital region of the females with a momentary thrust of the highly modified anal fin or gonopodium. Although it is generally known that in most of these thrusts the gonopodial tip never quite reaches the female, in many of these reports the assumption is made, or implied, that these thrusts are true copulations and result in the transfer of sperm from male to female.

In a recent study on reproductive behavior in two closely related poeciliid fishes, *Platy-poecilus maculatus* and *Xiphophorus hellerii* (Clark, Aronson & Gordon, 1948; in ms.), we found that this momentary thrust or jabbing response never resulted in insemination even when a definite contact of the gonopodium with the female's genital opening was attained. True copulation in *X. hellerii* and *P. maculatus* was determined to be a relatively prolonged act, lasting as much as 5.6 seconds, a period in which the male and female appear to be hooked together. Although these species have also been widely used in biological research for many years, these prolonged contacts had been reported briefly in only two previous studies. In view of this, we thought it possible that a prolonged type of contact in the guppy might have been overlooked, and that in this case also, the short but frequent thrusts, although part of the courtship pattern, were not involved in the actual insemination of the female.

In general there have been two views on the mechanism of copulation in the guppy.

Several investigators assumed that the momentary thrusts were the behavioral acts which resulted in insemination of the female. At the same time they realized that the gonopodium often never quite reached the female. Hence they explained the copulatory act by postulating that the sperm (in the form of relatively large capsules or spermatophores) are shot at the female genital opening by the gonopodium. Other investigators considered definite contact between the tip of the gonopodium and the genital orifice of the female necessary for sperm transfer.

This present report presents experimental evidence on some phases of sexual behavior in the guppy, particularly on the act of insemination. This evidence will serve to correct some of the erroneous conjectures. By a combination of observational methods on mating behavior, by utilizing isolated females, and by using a smear technique for the detection of sperm in the female after insemination, we have been able to determine and define the act of sperm transfer. Supplementary studies are also presented which we hope will contribute to the general understanding of sexual behavior in this and related species.

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graphic equipment for taking action pictures of the guppy during courtship. The drawings of the gonopodia were made by Mr. Donn E. Rosen. Mrs. Marie Holz-Tucker and Miss Madeline Levy prepared the histological material and assisted in the interpretation of it.

#### REVIEW OF THE LITERATURE.

Three stimulating papers dealing with the problem of sexual selection in fishes have included descriptions and discussions of sexual behavior in *Lebistes* (Breder & Coates, 1935; Noble, 1938; and Haskins & Haskins, 1949). Less detailed discussions of sexual behavior in the guppy are presented in the genetic studies of Schmidt (1920) and Winge (1922), in a study of sexual dimorphism by Purser (1941), in two reports on gonad morphology (Stepanek, 1928, and Vaupel, 1929), and in an investigation of female receptivity by Jaski (1939). In the popular aquarium literature, numerous shorter articles on the guppy include discussions of sexual behavior (Brüning, 1918; Ritscher, 1927; Rummel, 1932; Zahl, 1933; Dempster, 1947; Gordon, 1948; Matsuyama, 1949; Latham, 1949; and many others). Of special note is the review article on the guppy by Fraser-Brunner (1947).

#### ACT OF INSEMINATION.

Previous observers expressed a variety of ideas concerning the act of insemination. Some appear quite certain that they have witnessed it numerous times, while others claim it has rarely been seen (Stepanek, 1928) or never at all (Purser, 1941). Apparently, no observer has ever subjected his conclusions to an experimental test designed to show whether sperm transfer actually took place during the act he described. Many investigators, nevertheless, have presented some thought-provoking conjectures and it is a point of interest to review the various ideas concerning the copulatory act.

One of the earliest ideas on this subject was presented by Schmidt (1920) who suggested that an actual contact between the male and female is unnecessary. According to Schmidt, the male merely pursues the female, and when he approaches her he rotates his gonopodium so that the tip is close to her genital region. He then "actually discharges these balls [spermatophores] like shot, and, being glutinous, they easily attach themselves to the genital papilla of the female." Schmidt's hypothesis was accepted by Winge (1922), who in his extensive genetic investigations undoubtedly kept large breeding populations of guppies in his laboratory. It is easy to understand how this idea arose, since these non-contact thrusts of the gonopodium are the most frequently and easily seen of all the sexual behavior components. Indeed the authors of most of the reports in popular aquarium journals take it for granted that insemination is effected during this non-contact thrust. Recently Frazer-Brunner (1946) in his review article on the guppy stated: "Many people have watched Guppies

in the hope of seeing the act of coitus, but have never been rewarded; nor will they be, for there is none—at least in the form they expect to see. . . . When the male has effected a strategic position with regard to the female, usually approaching from the rear, he corrects the gonopodium and 'dive-bombs' the female. The cannon-ball-like spermatophores . . . are fired machine-gun fashion at the genital opening of the female. The tip of the gonopodium being flexible but controlled, considerable accuracy is obtained. . . . The whole process is carried through in a flash, and is hardly possible to observe under ordinary conditions. It appears that the tip of the gonopodium is brought as near as possible to the female without being introduced."

However, Vaupel (1929) regarded the hypothesis of Schmidt and Winge as "clearly incorrect." "My own observations," he stated, "corroborated by those of several experienced aquarists, have assured me that the gonopod undoubtedly makes a contact with the genital opening of the female." Zahl (1933) reached a similar conclusion that insemination occurs when the male "makes a quick plunge forward and upward, and for an instant establishes contact between the tip of the gonopodium and the genital orifice of the female. . . . It all happens with such lightning speed, that if one isn't a careful observer, the act may escape his notice." Breder & Coates (1935) observed that during courtship a male shows an "energetic movement of the exceedingly mobile gonopodium to the side next to the female. . . . Normal, healthy males seem to be almost continually active in this regard, interrupting it only for feeding, but without considerable observation this is about all that can be usually noted in a small aquarium with few fish. Prolonged observation will reveal, however, that eventually the male gives up this procedure and directs a rather violent thrust of the gonopodium toward the genital pore of the female. A momentary contact effects the transfer of the encapsulated spermatozoa." A similar view is shared by Haskins & Haskins (1949), who suggest that the "spermatophores are probably transferred along the male gonopodium to the genital pore of the female in one or more contacts . . . swift and of momentary duration." It is interesting to note that in the last mentioned article the authors report that prolonged contacts are the typical means of insemination in two closely related species, *Micropoecilia parva* and *Poecilia vivipara*.

Purser (1941) observed in detail the forward movements of the gonopodium during what we will refer to in this paper as the *swinging* and *thrusting* behaviors. However, he did not believe these to be acts of insemination but assumed that during coitus the gonopodium maintains the same position.

After the present study had been completed, the important paper by Stepanek

(1928), published in an obscure Czechoslovakian journal, was called to our attention. Here we found the only record in the literature of a prolonged gonopodial contact in the guppy.

Stepanek reports that after four years of observations on *Lebistes* he is convinced that spermatophores are ejaculated only during a prolonged contact of several seconds duration, during which period the gonopodium is inserted into the female. He witnessed this rare behavior only twice. He suggests that the terminal hook on the third ray of the gonopodium functions as a holdfast organ, and that when the tip of the gonopodium is inserted, the female cooperates by "closing over [presumably the sphincter of the genital aperture] and holding on." In this connection, however, the necessity of a terminal hook for insemination has been ruled out by recent amputation experiments of Sengün (1949). Stepanek's observations, however, are particularly significant because he is the only one to describe a long copulation and to suggest some degree of cooperation by the female. He appears to be the only author who has recognized the actual nature of insemination, as we believe we have validated it, in this fish.

#### BEHAVIOR OF THE FEMALE.

Aside from Stepanek's conclusions, and a questionable report by Jaski (1939), others agree on one point, namely, that there is a complete lack of cooperation on the part of the female guppy during the act of insemination (Breder & Coates, 1935; Fraser-Brunner 1947; Gordon, 1948; Haskins & Haskins, 1949; and most aquarists). Thus according to Breder & Coates (1935), "This actual transfer of material seems to occur only after the male has slipped up to the seemingly unsuspecting female. Not infrequently a male may be seen to court one fish and as she flees succeed in fertilizing another. . . . No females at any time have been observed to show other than escape reactions to the male attentions." Fraser-Brunner (1947) states, "All the sexuality seems to be on the part of the male. The female never appears to display the slightest interest, but generally tries to evade the attentions of her mate, or even drive him away." He goes on to say that the males put on a courtship display. "The female, however, seems never to be attracted by this, and when eventually the male makes his quick dart, it is to take her unawares." Similarly Haskins & Haskins (1949) report that the "female remains throughout an apparently entirely passive agent in the whole act of fertilization. There is normally no marked halting in swimming, and no evidence of cooperation in any active way, except possibly in the case of females which have been reared in isolation in the laboratory." The aquarists likewise say that the female constantly flees from the male during courtship.

On the other hand, Stepanek's suggestion

that the female responds by closing over the male gonopodium when it is inserted in her oviduct indicates a complementary reaction of the female during the act of insemination. Jaski (1939) proposed a rather unique theory concerning female receptivity. Based on extensive studies, Jaski claimed to have discovered a definite estrous cycle of about 4 to 6 days (in females kept at temperatures over 22° C.) which is dependent on ovarian secretions and which can be influenced by a hormone, copulin, secreted into the water by the male. According to him, the ovarian secretions influence the angle at which the female swims. As receptivity increases, he says, the female assumes an oblique position in relation to the surface of the water and her head is tilted upward. Receptivity is indicated by a change in angle of about 20°. This angle of the female's body, he suggests, may facilitate the copulatory act. Jaski's studies, although frequently referred to in recent aquarium literature, have never been confirmed and much of it is opposed by our findings.

#### RESUME OF THE MATING PATTERN.

Before a detailed analysis of our observations on the mating behavior of the guppy is attempted, it is desirable to present a general description of the mating activity and to define the terms adopted for the various behavioral patterns. The names given to many of these acts are taken from recent studies by Clark, Aronson & Gordon (1948; in ms.) and Schlosberg, Duncan & Daitch (1949) on the related poeciliid fishes *Platypoecilus maculatus*, the platyfish, and *Xiphophorus hellerii*, the swordtail.

When a pair of guppies is placed in an observation aquarium, the male may ignore the female for some time but more often he will persistently pursue the female and jab at or near her genital aperture with the tip of his extended gonopodium. During this period of pursuit the following types of behavior are observed:

1. *Gonopodial Swinging.* This term refers to the movement of the modified male anal fin or gonopodium which is coordinated with the forward motion of one of the pelvic fins. During "swinging," the gonopodium is swung laterally and forward. It continues to rotate until the tip points anteriorly and the edge of the gonopodium, which in the resting position is dorsal, now faces ventrally. Meanwhile the pelvic fin on the same side of the body is also brought forward and the gonopodium appears to be braced against the pelvic fin for a fraction of a second. The gonopodium and pelvic fin then return to their normal positions (Plates I and II). The whole "swing" is accomplished in much less than a second. Successive swings usually are made to alternate sides. The dorsal fin, unless already erect, is erected simultaneously with the forward swing of the gonopodium. The completion of several swings within one minute evidently indicates a sexually active

male. In some pairings, however, when the female is easily approached by the first advances of the male, copulations with sperm transfer may occur almost as soon as the male (or female) is introduced into the tank with the other fish and before there has been any swinging of the gonopodium. This indicates that swinging as such in the presence of the female is not necessary for insemination. Occasionally swinging is observed in isolated males, and hence it is possible that the highly active males mentioned above may have been swinging before their introduction into the mating tank.

2. *Body Curving.* The body of the male becomes strongly curved either in the form of a simple arc or "S"-like with the tail bent sharply to one side. This position may be held as long as three seconds during which time the male appears tense, and its body quivers. Body curving often starts while the male is up to six inches from the female and therefore, she is still within his field of vision. While this behavior is in progress the male generally moves toward the side of the female. A "thrust" often follows, and this in turn occasionally is followed by a short or long copulation (see below).

When a male first encounters a female, body curving is usually accompanied by a fully expanded caudal fin (a spectacular sight in highly colored and longtailed varieties). However, as the courtship progresses, the caudal fin is generally folded when the male approaches the female.

3. *Thrusting.*<sup>1</sup> In this behavior, the male swims alongside the female (either directly or following body curving), brings his gonopodium forward and to the side facing the female and thrusts at the genital area of the female in a quick jabbing movement (Plate III). At the same time the pelvic fin is brought forward as in the swinging behavior, suggesting that among other functions it serves as a buttress or prop for the gonopodium. The male usually thrusts at the female from a position slightly below her so that the thrust is actually an upward as well as a lateral or sidewise movement (Plates IV, V and VI).

Two types of "thrusting" are recognized and termed the non-contact thrust and the contact thrust. In the non-contact thrust, the male jabs his gonopodium close to the female genital region but does not touch her body. This is the most common type of thrusting observed. During contact thrusts the gonopodium of the male touches the female's body lightly for a momentary contact at her genital region.

4. *Short Copulations.* These are similar to contact thrusts except that (1) their duration of contact is slightly longer, at least 0.8 seconds, and (2) the act is associated with a stationary position of the female. The

short copulation is not easily distinguished from the contact thrust except after considerable experience in observation. It is usually preceded by body curving. Males may transfer hundreds of spermatophores to the female during a single copulation of this type.

5. *Long Copulations.* These resemble short copulations but last for a longer period of time. They average 1.3 to 2.4 seconds, but may be much longer. During a long copulation the male may circle under the body of the female while holding the tip of the gonopodium close to the genital aperture of the female, completing two entire circles before the fish swim apart. Long copulations are always preceded by body curving. Insemination frequently but not invariably takes place at this time.

#### RECEPTIVITY OF THE FEMALE.

During most of the courtship, the female swims away when the male approaches her. In many cases she swims rapidly and excitedly, fluttering up and down the side of the aquarium with her mouth rubbing the glass. A female often slaps her tail at the male that is sidling or thrusting at her. This "tail-slapping" behavior in fishes has been described for cichlids (Noble & Curtis, 1939; Aronson, 1949), monacanthids (Clark, 1950) and xiphophorins (Clark, Aronson & Gordon, in ms.). Sometimes a female will rest close to the bottom of the aquarium and although the male may persistently swim around her, he does not succeed in thrusting at her.

On some occasions when a male approaches in a "body curve," the female may suddenly become quiescent and come to an almost stationary position at a point away from the substratum. Short or long copulations generally follow. In a few instances, a female that was constantly fleeing the courting male gradually assumed the stationary receptive position. This change occurred after the male remained "face to face" with the female for a long period while blocking her forward movements by constantly maintaining this position in front of her. In each case a copulation followed. It thus appears that this stationary position assumed by the female is a sign of receptivity.

#### OBSERVATIONS ON MATING BEHAVIOR.

##### Material and Methods.

In all, 37 females were tested with 28 males in 56 observation periods which varied from one-half minute to three hours and 58 minutes in length. Observations were made on mature pairs of fish in two-gallon aquaria (25.0 cm. long  $\times$  16.5 cm. wide  $\times$  18.0 cm. high). All fish were kept in a greenhouse where the temperature varied between 25° and 28° C. Except for group I (see below), the incidence and duration of the various items of behavior observed were recorded on an electrically operated polygraph recording apparatus (see Clark, Aronson &

<sup>1</sup> In earlier studies on the platyfish and swordtail (Clark, Aronson & Gordon, 1948; 1949) we refer to this behavior as "jabbing" but have since adopted the use of the term "thrusting" from Schlosberg, Duncan & Daitch (1949).

Gordon, in ms.). All fish used were kept isolated for at least one day before their first pairing, and were immediately separated at the end of the test.

After all observations in which the male approached the female with a porrected gonopodium, the female was tested for the presence of sperm. The method used was developed by Clark, Aronson & Gordon (1948 and in ms.) in *Platylocilus maculatus* and *Xiphophorus hellerii* and was found by numerous experimental tests to be highly reliable. In the test, a fine pipette containing a drop of 0.8% saline solution is inserted well into the female gonaduct. By means of a rubber tube held in the experimenter's mouth at one end and ending in a pipette at the other, the drop is gently expelled into the genital duct and then the fluid sucked back into the pipette. The redrawn drop is then examined under the microscope. Negative sperm smears are rechecked at least one additional time. That the technique is adequate to test for sperm transfer in *Lebistes* is indicated by the records kept on 21 virgin females that were paired with males, smeared, then set aside and later checked for embryos or young. Of these, 17 gave negative sperm smears and in these negative cases no embryos or young were recovered. Of the four females with positive smears after mating observations, two produced young and two did not.

In all our observations we used either virgin females or females (taken from stock tanks) which were isolated until sperm could no longer be recovered from them by the smear technique. This usually took about one week.

Three sets of observations were made. In group I (Table 1), we used four special strains of fish, the females of which were virgins. The fish marked A were a golden

variety; B were albinos; C were of a cream strain; and D were wild type.<sup>2</sup> Group II (Table 2) were all of the wild type. All the females and males 1, 2, 5 and 6 were virgin fish reared in our laboratory from two broods born on March 31, 1949. In these broods the males were segregated from the females at the first signs of sexual dimorphism. Males 12 through 15 were taken from a stock of the Haskins' strain of wild type guppies. They had been raised with females and thus had opportunity for sexual experience prior to their use in our experiments. Group III (Table 3) consisted of mature guppies of unknown pedigrees taken from a large community tank. The males and females in this group probably all were sexually experienced. The females, although not used in observations until they gave negative sperm smears, probably had stored sperm and several dropped broods during the days they were used for observations.

In groups I and II, the female was introduced into the male's tank at the beginning of the observation. This technique of bringing the female to the male's quarters has been used by animal breeders (Winge, 1927; Beach, 1947) and was found to be very effective in our earlier experiments on *xiphophorin* fishes. In group III, the male was introduced into the female's tank. This method proved to be equally efficacious in the guppy.

#### Results.

The results of the three sets of observations are given in Tables 1, 2 and 3 and are summarized in Table 4.

*Short and Long Copulations.* During twelve of the 56 observation periods, the female was inseminated. In each of these cases at

<sup>2</sup> Dr. Caryl P. Haskins kindly supplied us with these strains.

TABLE 1.  
Observational Results on Group I (Virgin Females)<sup>1</sup>.

Female	Male	Length of Observation (in min.)	No. of Thrusts		No. of Copulations <sup>2</sup>		Smear	Embryos <sup>3</sup>
			Non-contact	Contact	Short	Long		
A-1	A-1	5	0	0	0	8	negative	absent
A-2	A-2	10	0	0	0	0		
A-2	A-3	2.5	2	0	0	1	positive	absent
B-1	B-1	15	7	3	0	0	negative	absent
C-1	C-1	6	0	0	0	0		
C-1	C-3	20	0	0	0	0		
C-2	C-2	20	0	0	0	0		
D-1	D-1	18	0	0	0	1	negative	absent
D-2	D-2	20	5	1	0	5	negative	absent
D-3	D-3	16	3	1	0	0	negative	
D-3	D-5 and 6	16	0 <sup>4</sup>	0 <sup>4</sup>	0	3	positive	present
D-4	D-4	16	0	0	0	0		
D-4	D-7 and 8	12	7 <sup>4</sup>	0 <sup>4</sup>	0	1	negative	absent
Total	9	15	176.5	24	5	0	19	
Mean			13.6	1.8	0.4	0	1.5	

<sup>1</sup> All observations were made on 12/7/48.

<sup>2</sup> All these females remained stationary when males approached and thrust. The resulting copulations were all of the long circling type.

<sup>3</sup> Females dissected and ova examined on 12/27/48 (20 days later).

<sup>4</sup> Male thrusts at each other not recorded.

TABLE 2.  
Observational Results on Group II (Virgin Females).

Female	Male	Date	Observation Length (in min.)	No. of Thrusts		No. of Copulations		Duration of Long Copulation (in sec.)	Smear Embryos <sup>1</sup>	No. of Swings S-curves per min.	No. of Swings S-curves per min. per min.	No. of Thrusts	
				Non-contact	Contact	Short	Long						
1	1 <sup>2</sup>	1/4/50	20	0	0	0	0			1.3			
1	1	1/4/50	14	0	0	0	0						
1	5 <sup>2</sup>	1/4/50	12	0	0	0	0						
1	6 <sup>2</sup>	1/4/50	12	0	0	0	0						
1	13	1/5/50	12	21	0	0	0	negative				1.8	
1	12	1/6/50	13	19	0	0	0	negative				1.5	
2	12	1/5/50	12	11	0	0	0	negative				0.9	
2	2	1/6/50	12	3	0	0	0	negative				0.3	
2	15	1/9/50	22	3	0	0	0	negative				0.1	
3	14	1/5/50	14	6	0	0	0	negative				0.4	
3	14	1/6/50	12	8	0	0	0	negative				0.7	
3	12	1/9/50	16	38	0	0	0	negative				2.4	
4	15	1/5/50	14	2	0	0	0	negative				0.1	
4	4	1/6/50	12	0	0	0	0	negative				0.0	
4	13	1/9/50	22	4	0	0	0	negative				0.2	
4	12	1/15/50	14	1	1	0	0	negative				0.1	
5	14	1/9/50	22	12	4	0	0	negative				0.7	
5	14	1/15/50	15	10	2	0	0	negative				0.8	
6	13	1/15/50	30	11	2	0	0	negative				0.4	
7	15	1/15/50	23	5	1	0	0	negative				0.3	
8	13	1/15/50	90	131	12	0	0	negative				1.6	
9	12	2/8/50	20	2	0	0	0	negative				0.1	
9	13	3/7/50	56	95	3	0	1	positive	2.0			2.7	
10	13	2/20/50	238	105	7	1	0	negative				0.5	
11	13	2/24/50	176	212	22	5	0	negative				1.3	
12	15	2/21/50	80	31	5	4	0	negative				0.5	
12	13	3/14/50	84	70	5	1	0	present				0.9	
13	13	3/2/50	80	67	5	2	0	positive ? (♀ died)				0.8	
13	13	3/8/50	22	8	5	0	1	negative	2.0			0.6	
15	13	3/10/50	60	22	12	1	0	positive ? (♀ died)				0.6	
16	13	3/13/50	60	26	8	0	0	negative				0.6	
Total	16	8	31 <sup>3</sup>	1269.0	789 <sup>4</sup>	93 <sup>4</sup>	15	2		1453 <sup>5</sup>	810 <sup>5</sup>	1.6	0.7
Mean			40.9	49.9 <sup>4</sup>	5.8 <sup>4</sup>	0.5	0.1			60.5 <sup>5</sup>	36.8 <sup>5</sup>	0.6	

<sup>1</sup> Females dissected 17-20 days after last observation.

<sup>2</sup> Virgin male.

<sup>3</sup> Total number of observations.

<sup>4</sup> Sum of last 16 observations only.

<sup>5</sup> Total and mean only for observations where figures are listed.

TABLE 3.  
Observational Results on Group III (Non-virgin Females).

Female	Male	Observation Date	Observation Length (in min.)	No. of Thrusts		No. of Copulations		Duration of Long Copulation (in sec.)	Smear	No. of Swings	No. of S-curves	No. of Thrusts
				Non-contact	Contact	Short	Long					
21	21	4/3/50	2	0	0	0	1		positive	0	2	0.0
22	22	4/3/50	10	0	0	1	0		positive	19	5	0.0
23	22	4/4/50	5	2	0	0	1	2.4	positive	17	4	0.4
24	21	4/4/50	3	0	0	0	1	1.5	positive	4	2	0.0
25	23	4/4/50	16	0	0	0	1	1.3	negative	24	7	0.0
26	24	4/4/50	5	4	0	0	1	2.0	negative	12	9	0.8
27	22	4/11/50	9	4	1	0	1	1.7	positive	24	14	0.6
28	21	4/11/50	26	3	3	0	0		negative	54	49	2.1
29	25	4/11/50	7	0	0	0	1	1.7	negative	0	1	0.0
30	24	4/11/50	1	0	0	1	0		positive	0	1	0.0
31	24	4/11/50	0.5	0	0	1	0		negative	0	1	0.0
32	24	4/11/50	30	5	4	0	0		negative	53	67	0.3
Total	12	5	114	18	8	3	7			207	162	14.9
Mean			9.5	1.5	0.7	0.3	0.6			17.3	13.5	1.2

<sup>1</sup> Total number of observations.

least one short or one long copulation was found to have taken place. The positive sperm smears contained thousands of sperm. In many cases, a slight pressure on the abdomen of the female released a small amount of milky white fluid from the genital aperture which could be sucked into a dry micropipette. This fluid, when examined under the microscope, was found to be teeming with highly motile spermatozoa. In four cases these heavy sperm smears were recovered after a single short copulation (Table 3, females 22 and 30) or after long copulations in which no thrusting, either contact or non-contact, had taken place (Table 3, females 21 and 24). Our data indicate that a short copulation is as effective as a long copulation for the transfer of sperm. Some individual males (Table 2, male 13; Table 3, male 22) inseminated females with both types of copulations during different observations, but no males engaged in both a short copulation and a long copulation in a single observation.

Even when experienced males were used (see females 4, 10, 11, 12 and 14 in Table 2, and females 25, 26, 29 and 31 in Table 3), short and long copulations sometimes did not effect sperm transfer. In two cases, as many as 5 and 8 long copulations did not result in insemination of the females (see Table 1). The eight virgins (Tables 1 and 2) that had negative smears after copulation were sacrificed 17 to 20 days later and none contained embryos.

*Non-contact and Contact Thrusts.* During 22 observation periods, the females received from 2 to 234 non-contact or contact thrusts without any short or long copulations taking place. None of these females was inseminated. Nine of these (Tables 1 and 2) were sacrificed 17 to 20 days later and none contained embryos.

Non-contact and contact thrusts were far more numerous than copulations. A total of 1,061 thrusts were recorded for the 56 observation periods, and most of these (about 88%) were of the non-contact type. Short copulations and long copulations occurred much less frequently—a total of only 14 of the former and 28 of the latter being recorded.

Swinging behavior, S-curving and thrusting were recorded in most of the observations for groups II and III. Since the observation periods were of unequal length, comparisons were made of the frequency per minute of these acts in observations where copulations did and did not take place. These comparisons (Table 5) show no significant differences, except in the case of the swinging behavior, which appeared to be significantly higher in observations where copulations occurred.

#### EXPERIMENTS ON PARTIAL AMPUTATIONS OF GONOPODIAL ELEMENTS.

The highly specialized parts of the gonopodium of poeciliid fishes have stimulated

TABLE 4.  
Summary of 56 Mating Observations.

Type of Thrust or Copulation	Number of Observations	Number of Observations Resulting in Insemination of the Female	Per cent. of Observations Resulting in Insemination of the Female
None	10	—	—
Non-contact and contact thrusts only	22	0	0
Short copulations <sup>1</sup>	9	5	55.6
Long copulations <sup>1</sup>	15	7	46.6

<sup>1</sup> Non-contact and contact thrusts also occurred in many of these observations.

considerable speculation as to their function, and numerous discussions on this topic have appeared in the literature. Brief reports by Langer (1913), Klemm (1924) and Stepanek (1928) indicate that sperm transfer does not occur when the gonopodium is first amputated. However, it has been established that *Lebistes* males may regenerate gonopodia which are structurally normal (Hopper, 1949) and are functional (Sengün, 1949). In an earlier study on the platyfish, *Platy-pocilus maculatus*, (Clark, Aronson & Gordon, 1949), it was found that after amputation of the minute tip of the gonopodium no regeneration took place, and males which previously were capable of inseminating females were no longer able either to copulate or to inseminate females. For that fish, it was concluded that this small complex tip was essentially a holdfast mechanism that operates during the copulatory act at which time the male appears to have a definite grip on the female. However, our observations on the act of insemination in the guppy show that this act is not always as definite as in the platyfish. Although copulation in the guppy may last as long as in the platyfish, the male and female do not appear "hooked" together. In view of these differences we decided to investigate the effect of the removal of various parts of the gonopodium on the inseminating ability of the male guppy. The following amputations were performed:

Group A—The major portion of the gonopodial hood<sup>3</sup> was removed from each of five males (Text-fig. 2A).

Group B—The distal quarter of rays 3, 4 and 5 was removed from each of five males (Text-fig. 2B).

Group C—The tip of rays 3, 4 and 5 which includes the hooks terminating ray 5 was removed from each of ten males (Text-fig. 2C).

Following the operation each male was paired with a female in a two-gallon aquarium. The females were checked for presence of sperm after two and six days.

The females used in these studies were not virgins. They were removed from a stock

tank two to three weeks prior to the experiment and isolated in individual two-gallon aquaria. In every case a negative sperm smear was obtained just prior to the test with an operated male.

The results of these three types of gonopodial amputations on the ability of males to inseminate females are presented in Table 6. They indicate that the gonopodial hood is not necessary for insemination, but that amputation of the distal portion of rays 3, 4, 5 and even the small distal tip prevents insemination from taking place for at least six days after the amputation. During this period little regeneration occurs. After one month the males of group C showed no significant regeneration of rays 3, 4 and 5.

#### HISTOLOGY OF THE GONOPODIAL HOOD.

Because of the unusual appearance of the gonopodial hood, a brief histological study of this structure was undertaken with the hope of gaining some understanding of its physiology in relation to copulation. The hood is shaped like an elongated trough, in which the concavity faces dorsally when the gonopodium is in the resting position (Pl. VII, Fig. 15, c.). Towards the distal end the concavity becomes rather narrow, and finally terminates as a short blind canal or caecum near the tip of the hood (Pl. VII, Figs. 16 and 17, ca.).

The outer or ventral surface of the hood, as well as the concavity or dorsal surface, is formed by a stratified epithelium two to four cells in thickness (Pl. VII, Figs. 15, 16 and 17, ep.). Occasional goblet cells are found in this epithelium, particularly towards the distal end. At the tip of the hood the epithelial layer becomes considerably thicker (Pl. VII, Fig. 17) and the deeper lying cells on the dorsal surface are long and spindle-shaped. The cytoplasm of some of the epithelial cells contains fine, weakly staining granules. These may be the oxyphilic granules described by Purser (1941).

Beneath the epithelial layer on the ventral side there is a thick, hyaline structure which stains a deep pink with haematoxylin and eosin (Pl. VII, Figs. 15, 16 and 17, co.). Purser (1941) has termed this the core. This layer is covered on both sides by thin core membranes (c.m.) which stain a deep purple in contrast to the pink of the compact

<sup>3</sup> The term "prepuce hood" is commonly used in the literature but we prefer to omit the word "prepuce" as it is redundant and may incorrectly imply an homology or relationship with the foreskin of the penis.



TABLE 5.  
A Comparison of Male Behavior in Observations in Which Copulations Did and Did Not Take Place.

Behavior	Observations With Copulations		Observations Without Copulations		Mean Difference Per Minute	"P Value"
	Number of Observations	Mean Frequency of Behavior Per Minute $\pm \sigma M$	Number of Observations	Mean Frequency of Behavior Per Minute $\pm \sigma M$		
Swinging	6	2.3 $\pm$ .39	16	1.2 $\pm$ .14	1.04	<0.01
S-curving	9	0.8 $\pm$ .17	13	0.6 $\pm$ .13	.22	0.3
Thrusting	9	0.9 $\pm$ .23	18	0.7 $\pm$ .16	.17	0.5

core. The latter curves inwardly around the edges of the trough. At this point the two core membranes fuse and extend from side to side just beneath the epithelium lining the trough. Under high magnification ( $950\times$ ), fine irregular lines can be seen in the compact layer, suggesting a fibrous composition. Cross sections of the core stained with eosin suggested a superficial resemblance to teleost scale. Moreover, when the epithelium and connective tissue of a fresh preparation were removed by immersion of the hood in a 1% solution of KOH, the core remained as a tough, transparent, curved sheet about the size of an average guppy scale. However, specific tests for calcium (Langeron's alizarin red S and von Kóssa's silver nitrate, Lillie, 1948) were negative. The Taenzer-Unna acid orcein test for the presence of compact elastic fibers (Lillie, 1948) also was negative. The core stained readily with methylene blue and took up the green (acid stain) of Masson's trichrome technique. The supposition is that we are dealing with a special type of dense, resilient, connective tissue in which all of the cellular elements are located at the periphery in the thin core membrane.

Between the core and the fused core membranes lining the concavity, there is a broad layer of loose connective tissue (Pl. VII, Figs. 15 and 16, *c.t.*) which is highly vascular and also contains bundles of longitudinally-running nerve fibers. Melanophores, when present, are found in this layer. Two large sinusoidal blood vessels run through the connective tissue layer, one on each side of the trough. Study of the living material reveals that the main right vessel (*r.b.v.*) carries the blood distally, while the main left vessel (*l.b.v.*) carries the blood in a proximal direction. Two major anastomoses occur between these vessels, one near the tip and the other near the middle of the hood.

Whole mounts of the hood stained with methylene blue (Pl. VII, Fig. 18) reveal several compact bundles of longitudinally-running nerve fibers, and at the tip a rather extensive plexus of fine nerve fibers. In cross sections stained with methylene blue, some of these fine fibers could be seen terminating in small bulbs suggestive of nerve endings. A whole mount stained with the gold chloride method of Ranvier (McClung, 1929) verified the extensive innervation of the gonopodial hood.

#### MATING ACTIVITY AMONG GUPPIES IN COMMUNITY TANKS.

In several previous studies on *Lebistes*, Breder & Coates (1935), Fraser-Brunner (1947), and Haskins & Haskins (1949) implied that male guppies copulate with and inseminate females at a very high frequency, perhaps hundreds of times daily. In view of our finding that copulation actually is a rather infrequent event in test pairings, we set out to determine the frequency of such behavior among fish in community tanks.

TABLE 6.  
The Effects of Partial Amputations of the Gonopodia on  
the Inseminating Ability of Male Guppies.

Group	Male	Part of Gonopodium Removed	Sperm Smears on Female	
			After 2 Days	After 6 Days
A	$\left. \begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5^1 \end{array} \right\}$	hood	positive	—
			negative	negative
			positive	—
			negative	positive
			positive	—
B	6 through 10	distal quarter of gonopodium	all negative	all negative
C	11 through 20	distal tip of gonopodium including hook on ray 5	all negative	all negative

<sup>1</sup> This male had a long copulation with the female about 10 minutes after pairing. The female was smeared a few minutes later and found to be heavily inseminated.

This could only be estimated by indirect methods.

First, it was necessary to find how many days following an insemination a positive smear could be obtained. A single effective copulation results in the transfer of hundreds of spermatophores. When a sperm smear is taken within a few hours after an effective insemination, the fluid in the female oviduct is macroscopically "milky" in appearance. This milky fluid can no longer be recovered from females two days after copulation. At this time the sperm smear appears clear although many hundreds of spermatozoa are still seen upon microscopic examination, although they are not nearly as dense as in a "milky smear." After seven days, the number of sperm in the smear is considerably less in most cases, and occasionally the smear is entirely devoid of sperm. By the eleventh and fourteenth day, few, if any, sperm are recovered.<sup>4</sup> Our data are summarized in Table 7.

In the light of this schedule of smear changes on successive days after insemination, random samples of females were now taken from stock tanks for examination. Each tank contained many males. Smears were made in all cases shortly after females were removed from the tanks. The results of this examination are summarized in Table 8. Only 26 females (out of 54) contained sufficient sperms to indicate a recent insemination, and only six of these appeared to have been inseminated within the preceding day. More than half of the females tested (28) appeared not to have been inseminated for more than a week.

#### MODIFICATION OF THE SMEAR TECHNIQUE FOR THE DETECTION OF SPERM.

As indicated above, it is difficult to recover sperm two weeks after insemination by the ordinary smear technique. A more radical

<sup>4</sup> The reduction in the amount of sperm recovered in smears on successive days after insemination may be due to two factors, namely, the ingestion of sperm by ameoboid cells in the ovary, as found in *Glaridichthys* (Philippi, 1909), and the concentration of sperm in the folds of the oviduct reported by Philippi (1909) in *Glaridichthys* and in the folds of the ovary as seen in the histological studies of Winge (1922) and Stepanek (1928) on *Lebistes*.

method was required. Ten females tested on April 28, 1950, (Table 8) with positive sperm smears were retested by the smear technique on May 11, 1950, after 13 days of isolation and all gave negative smears (two smears were taken from each female). These same females were then smeared a third time on the same day but this time the drop of fluid was blown into the genital tract, and the tip of the micropipette was rubbed against the folds of the oviduct a few times before the drop was sucked back into the micropipette. The smears obtained in this manner gave positive signs of sperm in six of the ten females, less than ten sperm in four cases, about 50 in one, and about 150 in another.

#### DISCUSSION.

##### Female Behavior.

One of the most striking aspects of the act of sperm transfer is the receptive stance of the female at the moment of contact with the male. Although at most other times the female flees from the male, there are periods—which may be very brief—when the female halts in her agitated swimming and remains stationary while the male copulates with her.

TABLE 7.  
Sperm Recovery from Female Guppies.<sup>1</sup>

Female	Number of Sperm in Smear on Varying Days After Insemination			
	1 Day	7 Days	11 Days	14 Days
1A		40		10
3A		200		0
4A		20		0
5A		50		0
9		0		
12A		300		10
13A		500		2
21			0	
22			0	
23	>1000		80	6
24	>1000		0	
27	>1000	30	0	
30	>1000	10	0	
31				0

<sup>1</sup> Numbers over 10 are approximations of the number of sperm in the saline smear drop.

TABLE 8.  
Sperm Recovery from Females Kept with Males in Community Tanks.

Date	Tank Size at Water Level		Approximate Number of Fish in Tank <sup>2</sup>	Number of Females Tested	Number of females having smears with:			No Sperm	
	Dimensions in cm. <sup>1</sup>	Capacity in liters			Thousands of Sperm (Milky Drop)	Handreds of Sperm (Clear Drop)	Few Dozens of Sperm		Less than 10 Sperm
2/25/50	38 × 31 × 20	23.6	38	19	3	6	2	3	5
3/17/50	460 × 76 × 41	1430.0	>500	20	2	4	2	1	11
4/28/50	182 × 81 × 66	970.0	>200	15	1	1	5	3	5
			Total	54	6	11	9	7	21
					1 day	2 to 7 days			> 7 days
					Estimated last insemination before smear				

<sup>1</sup> Measurements given in length, width and height respectively.

<sup>2</sup> Figure given is for adults only; numerous young were also present.

We have observed this receptive behavior not only in virgin females placed together with mature males for the first time (groups I and II), but also in sexually experienced females, raised to adulthood in the presence of males except for the short isolation period of approximately two weeks before observations were begun on them (group III). In rodents and other mammals where sexual behavior has been studied most extensively, female mating behavior generally consists of one or more *positive* overt acts (Young, 1941). In the guppy the female mating pattern is primarily a *negative* response; that is, remaining stationary and failing to move away from the male. This, however, does not lessen the significance of this behavior. This situation is by no means unique for the guppy. Thus in the platyfish and in the swordtail an important feature of the female pattern is a similar failure to move away from the male at crucial periods. In these fish, however, this stance of the females is not as pronounced as in the guppy and once contact is made the copulating pair may move along, particularly during the more protracted contacts of the swordtail (Clark, Aronson & Gordon, in press).

In *Poecilia vivipara* and *Micropoecilia parae*, Haskins & Haskins (1949) state that the female responds to the actual gonopodial contact of the male by halting momentarily in her swimming and thereby rotating her body slightly towards the side from which the male has approached. In the leopard frog, *Rana pipiens*, and the green frog, *Rana clamitans*, receptivity is likewise a *negative* response (Noble & Aronson, 1942; Aronson, 1943). In the unovulated or spent female frog, the audible warning croak is the stimulus which causes the male to release his clasp. The ovulated female (i.e., one ready to lay her eggs) does not emit the warning croak when clasped by the sexually active male. This is clearly a receptive response. In sheep, according to McKenzie & Terrill (1937), there are no reliable indications of heat until the estrous female is teased by the ram whereupon she will stand and allow the ram to mount. When not in heat, the ewe will move away quickly when teasing action starts. From the review of Young (1941) it is clear that in many other mammals the failure to move away or resist the approaches of the male is the receptive behavior of the female. This behavior is often discriminative and selective.

In contrast to our results, most of the published discussions on sexual behavior in the guppy state that during the courtship the female constantly flees from the male, and when he does contact her, it is by taking her "unawares." From our results, it would seem that none of these authors had seen or recognized a true copulatory act. An exception to this general misconception concerning the behavior of the female during copulation was expressed somewhat indirectly by Stepanek (1928) who believed it possible

that the female responded to the male by closing [the sphincter of the genital aperture?] over the inserted tip of the gonopodium. It is possible that this theory is based on observations of the receptive stance of the female.

Certain of Jaski's statements (1939) concerning the sexual behavior of the female guppy may now be considered. Jaski described an estrous cycle in the guppy which is reflected by changes in the angle at which the female swims. In view of Jaski's reports we particularly took notice of the swimming and resting angle of those females that did and those that did not copulate. However, we did not observe any significant correlations between these features of behavior.

Jaski also reported that virgin females first introduced into aquaria with males did not come into estrus for 3 to 4 days. Hence, according to him, it was practically impossible to mate them for several days due to their non-receptivity. Our observational records show that virgin females may readily mate on the day they are first paired with males (see females A-2 and D-3 in group I; females 13 and 15 in group II). Three other virgin females that were paired overnight to test fertility of males also became inseminated in this time. In fact, some of our females (group I) copulated within a few minutes after their first contact with males. Before their first pairing, our virgin females were not exposed to water in which male guppies had lived and hence could not have been under the influence of what Jaski calls "copulin," the female-stimulating hormone supposedly secreted by the male guppy. However, we followed the standard aquarium practice of using "fish-conditioned water" obtained from large stock tanks of the cichlid fish *Tilapia macrocephala*. It is well known among aquarists that when some aquarium fish are introduced into tap water or standing water in which no plants or animals have lived for some time, they may suffer from a shock-like condition. Sometimes they survive, but it may often take a day or more to recover completely. It is possible that Jaski's results may be explained in part by a generalized water conditioning effect instead of the specific sexual secretion that he postulates. Dr. Breder states (personal communication) that he tried to repeat some of Jaski's experiments. Breder used tap water which he conditioned with plants before introducing the fish. Under such circumstances the fish showed no signs of distress when first placed in the aquaria used for his tests, and the females showed no cyclical variation in the swimming angle as reported by Jaski. Breder states further that, in his experience, only sick guppies assume other than the normal resting or swimming position.

Our studies suggest that some females are more receptive at some times than others. In some of our observations the female consistently swam away from the male or rested

on the bottom of the tank so that the male could not bring the gonopodium close to her genital region at any time in the entire observation period, which in several cases (group II) lasted for over an hour. In other cases, the constant fleeing behavior of the female appeared to prevent copulation even though considerable jabbing took place. In still other cases, copulations occurred within one or two minutes after the pair had been placed together. Whether receptivity in the female guppy is a cyclical physiological phenomenon, or a state which results from more immediate environmental conditions, or both, remains to be determined.

#### Male Behavior.

It can be seen from this study that the male guppy inseminates the female only during the copulatory act which involves a definite contact between the tip of the male gonopodium and the female genital region. During these copulations the female remains stationary while the male pushes against her with his gonopodium. The contact may be very brief, or may last several seconds. The male's numerous non-contact jabs and contact jabs at a fleeting female, so commonly observed in aquaria of mixed sexes of guppies, are not acts of insemination as reported by many authors.

Breder & Coates (1935) raised the question why frequent inseminations take place in the guppy, especially when it is known that isolated females, for months after a single insemination, may continue to drop broods—as many as eight, according to Winge (1922, 1937). Actually, as we have shown by both observational methods and by smear tests of females from community tanks, actual inseminations occur relatively infrequently and the "large wastage of sperm" is not as great as these authors suspected. In reality, females are rarely inseminated more than a few times a week if that often (Table 7). Relative to the number of young produced, the amount of sperm expended by the guppy may well be no greater than in many other vertebrate species.

A limited number of reinseminations may have considerable adaptive significance. When poeciliid females are isolated shortly after one or more inseminations, the size of brood tends to decrease in the ensuing series of roughly monthly parturitions (Breder & Coates, 1932; Breder, 1934). On the other hand, in females that are constantly being reinseminated, the size of brood remains large. Moreover, according to Philippi (1909), Van Oordt (1928) and Breder (1934), isolated females may temporarily discontinue dropping broods, especially during winter months.

The first four males in group II (nos. 1, 2, 5 and 6) were sexually inexperienced males that had been segregated from females at the time their gonopodia started to differentiate. As can be seen from Table 2, these

males did not thrust or copulate. Breder & Coates (1935), Noble (1938), Haskins & Haskins (1949), and Clark, Aronson & Gordon (In ms.) have all emphasized the role of learning in the selection of mates. It seems plausible, therefore, that the failure of these virgin males to thrust or copulate may be attributed to their lack of previous courtship and copulatory experience.

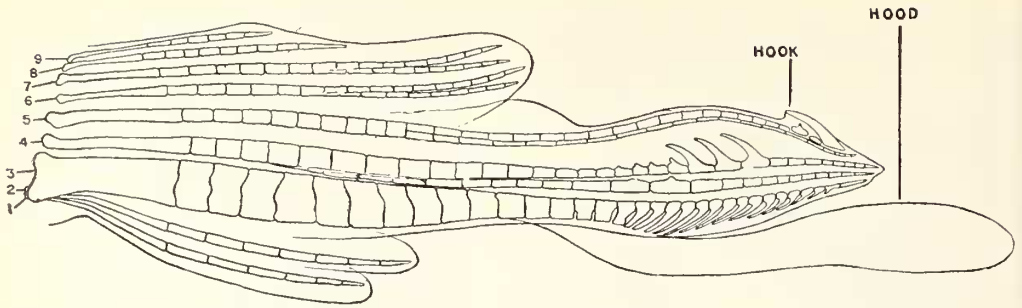
Haskins & Haskins (1949), in a revealing study of sexual selection in the guppy, introduced experienced male *Lebistes* into a tank containing one female *Lebistes*, one female *Micropoecilia* and one female *Poecilia*. Gonopodial contacts were counted and it was found that shortly after their introduction, the greatest percentage of the contacts were with the *Micropoecilia* female. After being left together for one week, the contacts were almost all with the *Lebistes* female. In a similar experiment these authors showed that this adjustment to the *Lebistes* female is completed in three days. The discrimination involved, according to Haskins & Haskins, is based essentially on the behavior of the males, and gives some evidence of being a learned reaction. It seems fairly clear from their descriptions of the mating process that these authors were really counting jabs, and that copulations did not occur during their observation periods. This is indicated especially by our finding that fish in community tanks are not likely to copulate within an even lengthy observation period. However, the counting of jabs rather than actual inseminations does not necessarily detract from the importance of the findings of both Breder & Coates (1935) and Haskins & Haskins (1949) relative to sexual discrimination in the guppy, as there may very well be a high correlation between courtship behavior and ability and opportunity for copulation. Our data on gonopodium swinging suggest such a correlation. In observations where copulations occurred, the mean number of swings was significantly higher than in observations in which copulations did not occur. On the other hand, significant differences were not found in the mean frequencies for S-curving and thrusting between observations where copulations did and did not occur. It should be noted, however, that the relationship between courtship and copulation is made somewhat obscure by the fact that the most highly excited males will copulate with few or no preliminary acts. Thus it seems that the sexually least excited as well as the most excited males will have the lowest courtship scores while the high scores for these activities are for the most part a reflection of the responsiveness of an intermediate group of males. Since in the previously mentioned studies on sexual selection in the guppy, thrusting behavior was used as the major criterion for a differential reaction to the opposite sex, and since we have shown that thrusting does not result in insemination, it becomes increasingly important for future studies to determine

more exactly the relation of thrusting to copulation.

#### The Function of the Gonopodium.

Just how the gonopodium of poeciliid fishes functions in the transfer of spermatophores from the male to the female during copulation has long been a subject for speculation. It has been suggested that in the guppy the pelvic fins are rotated forward with the gonopodium during "coitus" (Purser, 1941) in such a manner that the tips of the pelvic fins (which in the male are modified elongations of the second rays) are slipped into the hood, thus forming a tube-like structure through which the sperm are shot out at the female (Fraser-Brunner, 1947). Vaupel (1929) proposed that the hood may be inserted and then expanded within the female genital aperture, thus permitting the entrance of spermatophores. Christman (1928) reported that during gonopodial contact only one pelvic fin is brought forward. Our observations confirm those of Christman. We have found that during swinging, thrusting, and copulation, one pelvic fin is brought forward and that it is always the one on the side towards which the gonopodium is swung (Plate V). Thus an enclosure is formed into which the spermatophores are ejaculated and directed towards the female. The hood is too small and transparent to be seen readily during behavioral observations, but our photographs of guppies while swinging and thrusting, taken with an electric flash unit (Plates I through V) show the hood dangling loosely. In view of its soft and flabby structure it is therefore very doubtful that during copulation the hood either holds the tip of the pelvic fin as Fraser-Brunner reported (1947) or is inserted into the genital opening as Vaupel suggested (1929). Moreover, our data on amputation and Sengün's (1949) regeneration experiments show clearly that this structure is not necessary for effective insemination. The histological examination of the hood has demonstrated a large number of nerve fibers extending to the tip where there is an extensive nerve plexus. Since the structure contains no muscles, and few glands, it is presumed that these nerves are mostly sensory, and that the gonopodial hood is primarily a sensory organ. Although our experiment revealed that amputation of the hood does not interfere with insemination, it should be noted that we used only sexually experienced males. It may be of considerable interest to repeat the above experiment using inexperienced males and observational procedures.

An examination of the morphology of the gonopodium (Text-fig. 1) reveals that the most basic and elaborate components are rays 3, 4 and 5. Our experimental results after ablating the distal ends of these rays confirm the findings of Sengün and the views expressed by most authors, namely, that these rays are indispensable for sperm transfer. Stepanek stated that the distal com-

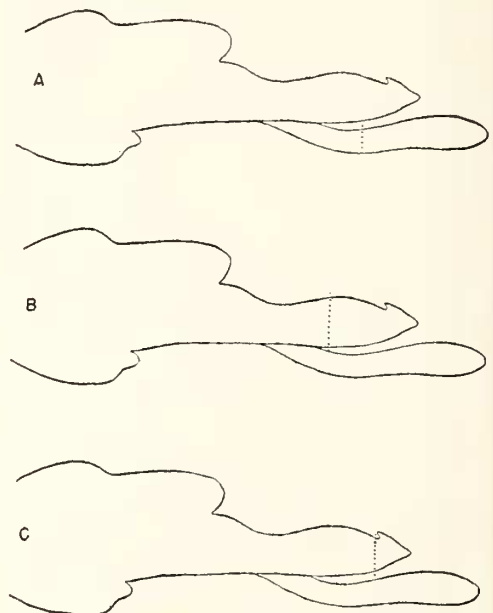


TEXT-FIG. 1. Gonopodium of a mature male *Lebistes reticulatus*.  $\times 22$ .

ponents, especially the hook on ray 5, was necessary for the grip of the male on the female, which he believed occurred during copulation. Sengün, however, showed that males with abnormally regenerated gonopodia and lacking this hook, are able to inseminate females. Our results indicate that the absence of the tip of rays 3, 4 and 5 which includes this hook and other modified serrae-like ray segments may prevent copulation. But as Sengün's studies indicate, the gonopodia may again become functional after an abnormal regeneration of this region.

In *Platypoecilus maculatus* and *Xiphophorus hellerii*, the tip of the gonopodium is differentiated into an arrowhead-shaped structure. It consists of hooks and serrae highly suggestive of a holdfast organ; the hood, however, is lacking. In these species insemination occurs only during a pronounced copulatory act during which the pair appear hooked together and may even swim around the tank in close contact. Copulation in these fishes ends in a pronounced snap-like break as the fishes appear to pull away forcibly from each other (Clark, Aronson & Gordon, In ms.). In *Lebistes* the hooks and serrae on the tip of the gonopodium are not nearly so prominent. Our observations lead us to believe that during copulation the male and female guppies are not hooked together as in the platyfish and the swordtail; rather, contact is maintained by the male who actively swims and pushes against the stationary female. This is most easily observed during the long copulations. Moreover, since the male and female are not hooked, the termination of copulation is not accompanied by any sharp, snap-like break. It is not surprising therefore that copulation in the guppy is not usually as pronounced and as easily recognized as in either the platyfish or swordtail. Dr. Haskins, in a personal communication, has informed us of an unusual case where a pair of guppies were found attached to each other in one of his aquaria. At the time they were discovered the male was dead and the tip of his gonopodium was firmly hooked to the genital region of the female (Plate VII, Fig. 14). We believe that the actual attachment which occurred here represents an abnormal situation resulting, perhaps, from an atypical gonopodium.

Gonopodial function evidently shows some important differences among viviparous cyprinodonts. In *Horaichthys* the gonopodium-like anal fin is greatly modified, particularly the distal segments of rays 4 and 5 (Kulkarni, 1940). However, these modifications are of a very different nature from *X. hellerii*, *P. maculatus* or *L. reticulatus*. Horn-like, spoon-shaped, conical, and attenuated processes are conspicuously developed, and except for a small hyaline recurved hook on the inner wall of the tubular portion of ray 5, there are no conspicuous hooks or serrae. Kulkarni observed mating in this fish. By examining females for sperm (which are packed in spermatophores embedded in the tissue around the female genital papilla) after his observations, he discovered that spermatophores are transferred to the female during a momentary contact. As the male "approaches his mate, he lashes out the gonopodium sideways almost at right angles to his body and strikes its terminal end



TEXT-FIG. 2. Outline drawings of *Lebistes* gonopodia. Broken lines indicate levels at which amputations were made. A, B, and C correspond to groups designated in Table 6.

against her genital opening" in what we would probably term a contact thrust or possibly a short copulation.

It thus appears that in the various cyprinodonts the gonopodia function in quite different fashions. Their customary designation as "intromittent organs" is misleading. The highly differentiated and complex gonopodia of most cyprinodontid fishes are indeed remarkable structures. Although gonopodial morphology has been studied in great detail, especially for taxonomic (Regan, 1913) and genetic (Sengün, 1949; Gordon & Rosen, 1951) purposes, little is known concerning the reproductive behavior of most of these cyprinodonts. However, on the basis of our present knowledge one could expect to find a definite correlation between gonopodial structure and function. Hence further comparative studies of cyprinodont sexual behavior and gonopodial morphology coupled with experimental methods involving various types of gonopodial ablations should reveal far more valuable information.

#### SUMMARY AND CONCLUSIONS.

1. A review of the scientific and popular literature reveals many interesting and controversial ideas concerning the mechanisms of sexual behavior of the guppy, particularly in regard to the act of insemination.

2. In a series of observational studies and experiments, various courtship patterns were analyzed, particularly the behavior of the male which we called gonopodial swinging, body curving, thrusting and copulation.

3. By the use of a genital smear technique, it was possible to detect the presence of sperm in females several days after insemination. By taking smears on females immediately after observations it was learned that actual inseminations are relatively infrequent. Inseminations occur during definite and recognizable types of contacts (copulations) between the male and female when the latter specifically halts in her swimming. Inseminations were not effected during the commonly observed non-contact and momentary contact thrusts.

4. The action and function of the gonopodium was analyzed. During swinging, thrusting and copulation the gonopodium is brought forward and to one side, together with a forward movement of one of the pelvic fins. The gonopodial hood is not necessary for insemination. The presence of large numbers of nerve fibers and the extensive plexus at the tip of the gonopodial hood suggests that it serves primarily in a sensory capacity. The absence of the distal segments of rays 3, 4 and 5 hinders and may completely prevent sperm-transfer to the female.

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## EXPLANATION OF THE PLATES.

## PLATE I.

Action of the gonopodium at the beginning of swinging behavior.

- Fig. 1. Gonopodium in resting position (pointing caudally). The arrow points to the gonopodial hood.
- Fig. 2. Gonopodium at the beginning of a "swing" is moving ventrally away from the body of the fish.
- Fig. 3. Gonopodium moving forward and to left side; note position of left pelvic fin which is also moving forward. The dorsal fin often remains folded at this stage.

## PLATE II.

Action of the gonopodium at the height of swinging behavior.

- Fig. 4. The gonopodium is now forward and on the left side of the fish. The left pelvic fin is also forward and the dorsal fin is erected. Arrow points to gonopodial hood.
- Fig. 5. A slightly more advanced stage of the preceding picture. The pelvic fin is completely forward.
- Fig. 6. The gonopodium is far forward. It appears braced against the pelvic fin. The gonopodial hood (arrow) still dangles loosely on the dorsal margin of the gonopodium now that the latter has been swung through a 180° arc. The body of the fish is arching upwards. This is a picture of a swing to the right.
- Fig. 7. The peak of a swing on the left side of the fish showing the body twisted into an "S" shape.

## PLATE III.

Thrusting behavior.

- Fig. 8. A male (left) approaching a female from above and thrusting at her.
- Fig. 9. A male (left) approaching a female from the side and thrusting.

## PLATE IV.

Thrusting behavior.

- Fig. 10. A male (right) in position to make a contact thrust. Female's body is straight but male's body is curved and tilted toward female.

- Fig. 11. A male (right) in position just before or after a contact thrust. The female is tilted away from the male but his gonopodium is still within reach of her genital region.

## PLATE V.

- Fig. 12. A male approaching the right side of the female for a thrust. Gonopodium and left pelvic fin are being brought forward in the same manner as in swinging behavior.

## PLATE VI.

- Fig. 13. The same male as in Plate V, approaching the other side of the female. The gonopodium and pelvic fin are completely forward. Note the gonopodial hood (arrow) which in this picture appears dark.

## PLATE VII.

- Fig. 14. Unusual case where the gonopodium of a dead male was found firmly hooked to the genital region of the female. Courtesy of Dr. Caryl P. Haskins.
- Fig. 15. Transverse section through the proximal portion of the gonopodial hood. Haematoxylin and eosin  $\times 410$ .
- Fig. 16. Transverse section through the middle of the gonopodial hood. Haematoxylin and eosin  $\times 410$ .
- Fig. 17. Transverse section through the distal tip of the gonopodial hood. Haematoxylin and eosin  $\times 480$ .
- Fig. 18. Whole mount of tip of gonopodial hood showing nerve plexus. Large black dots are melanophores. Methylene blue  $\times 280$ .

## Abbreviations:

- c. = concavity of hood  
 ca. = caecum  
 ep. = epithelium  
 co. = core  
 c.m. = core membrane  
 c.t. = connective tissue layer  
 l.b.v. = major left blood vessel (carries blood proximally)  
 r.b.v. = major right blood vessel (carries blood distally)  
 m. = melanophore